REMARKS

By this Amendment, Applicants herein provide an amended set of claims which clarify points raised by the Examiner without affecting the scope of the claims and which now overcome all outstanding rejections. Following these amendments, claims 1-41 remain pending in the application. For reasons as stated below, Applicants submit that the present claims now overcome all previous objections and have been placed in condition for immediate allowance.

In the Official Action, the Examiner noted that there were minor problems with the Sequence Listing, and these minor problems are now overcome in the diskette and new sequence listing attached hereto. The specification has also been amended as requested to refer to the proper sequence ID numbers, and thus this objection is respectfully traversed.

In the Official Action, at Page 3, the Examiner stated that "all claims are being read as product by process claims, wherein the claimed product may be produced by a different process that results in the same or equivalent product." The basis of this statement is unclear since most of the claims in the present application are method claims and not product claims. The specific objections to the claims by the Examiner will be addressed below.

In the Official Action, the Examiner objected to Claims 5 and 39 on the basis of the use in certain cases of abbreviations. Applicants have now amended Claims 5 and 39 to include the name of the abbreviated adhesin wherever possible, but in some cases (e.g., Sdr, CNA, MHC-II, etc.), these are the proteins' actual names and these are not abbreviations. For example, The term "MHC-II" refers to cell-surface molecules

that are responsible for rapid graft rejections and are required for antigen presentation to T-cells. Accordingly, claims where this antigen has been introduced do not include anything other than "MHC-II", as shown for Example in U.S. Pat. No. 5,648,240, Claim 1. The term "Sdr protein" is also recognized in the art as a protein which has a repeating sequence of serine and aspartic acid (which are identified as "S" and "D", respectively, in IUPAC nomenclature) having sufficient length to allow efficient expression of the ligand binding domain region A on the cell surface (such as disclosed at page 30 of the original specification). Accordingly, the proteins designated as "SdrC, SdrD, SdrE, SdrF, SdrG and SdrH" are also not abbreviations but are in fact the actual names of these proteins.

In the Official Action, the Examiner objected to Claims 1, 4,5, 38-40, 11, 15-16, 23, 26-28, 29, and 30-40 under 35 U.S.C. § 112 in that it the Examiner appeared to be somewhat uncertain as to the nature of the claims. For example, the Examiner seemed to indicate that the claims were directed to a "claimed genus of immunoglobulins to any clumping factor protein, any Sdr protein, and a second adhesion [sic] from any source." See Official Action at page 4. Contrary to the Examiner's impression, the present claims (before and after amendments) relate to a method of obtaining a human immunoglobulin composition that has a high titer to the clumping factor A adhesin. This immunoglobulin composition is obtained by selecting donor human blood or plasma which has a level of antibody titer to staphylococcal ClfA in an amount than is higher than that observed in pooled immunoglobulin products from unselected human donors, such as Baxter's Gammagard®, which are well known to one skilled in this art.. In particular, the present invention is carried out by selecting for donors with the high level

of antibody titer to staphylococcal ClfA, either by screening donors or by stimulating donors and then selecting the donors who after stimulation evidence a higher ClfA antibody titer.

In the relevant dependent claims, the method has an added step in that donors will be selected who have a titer to a second staphylococcal adhesin which is also higher than the level of antibody titer to that adhesin than will be found in the conventional immunoglobulin obtained from unselected donors, e.g., Baxter's Gammagard®. Accordingly, the method wherein a second staphylococcal adhesin is used to screen donor plasma will result in a donor plasma composition that has a higher antibody titer to two different staphylococcal adhesins, namely ClfA and the second adhesin as set forth in the claims. On the other hand, the basic method without the added step of screening for a second adhesin results in a selected immunoglobulin composition with a high titer to ClfA only. Accordingly, the dependent claims with the additional screening steps are proper claims and add another limitation to the basic process.

In the Official Action, the Examiner had numerous queries with regard to the nature of claims, and also asserted a variety of miscellaneous rejections under 35 U.S.C. §112 in association with these queries. To the extent that the foregoing does not alleviate the Examiner's uncertainty regarding the claims, Applicants address these objections as follows:

With regard to the source of the clumping factor A, this source is from Staphylococcus as the Examiner recognized as one of the possibilities. Applicants have now amended Claim 1 to make this clear.

With regard to the question of whether the method steps clearly allow one skilled in the art to practice the invention, these steps indeed allow the invention to be practiced, and the claims allow one to obtain a human donor immunoglobulin having a titer to ClfA higher than that found in unselected pooled intravenous immunoglobulin products obtained from unselected donors such as Baxter's Gammagard®, which are well known to one skilled in this art. As reflected in the present claims, the present invention relates to methods and compositions which constitute a distinct improvement over prior art products which have long been on the market which are made from pooled intravenous immunoglobulin obtained from donors who have not been selected for high titers of antibodies to staphylococcal ClfA. These pooled intravenous immunoglobulin products (also referred to as an "IVIG" product) are well known to one skilled in the art, and include products such as Gammagard® from Baxter as was referred to at pages 71 and 72 of the specification and Table 3. Baxter's Gammagard® is a normal unselected IGIV product which reflects pooled intravenous immunoglobulin obtained from unselected human donors, and the present invention is a distinct improvement over unselected IGIV because it will have a higher titer to the ClfA protein from *S. aureus*. Normal unselected IGIV is thus a standard product and is well known to one skilled in this art.

As reflected in Table 3 at page 72 of Applicants' specification, the present invention constitutes a distinct improvement over the unselected IGIV product obtained from unselected human donors in that this method results in an immunoglobulin composition having a higher titer to ClfA from *S. aureus*. This is reflected in Table 3 at page 72 of Applicants' specification. As shown in that Table, the selected human donor

immunoglobulin composition of the present invention (SA-IVIG MS502) had a high-titer against ClfA with a total ClfA content of 2.29 Units/mg as opposed to only 0.2 Units/mg in the unselected pooled human donor immunoglobulin. As shown in the experimental results provided in Applicants' specification at pages 72-74, it was clear that the therapeutic administration of the selected high-titer human donor immunoglobulin of the present invention provides a significant and effective treatment of staphylococcal infection as compared to the normal unselected pooled intravenous immunoglobulin product such as Baxter's Gammagard®. Accordingly, the claims as amended would be clear to one skilled in the art and readily allow one to practice the present invention.

With regard to the use of the term "A domain", the central aspect of the present invention is the ability to screen donors for a high titer of antibodies to a staphylococcal adhesin, which in Claim 1 is ClfA. In the basic screening method, donors are thus screened for high titers of antibodies to ClfA. However, Applicants have discovered that this screening process can be carried out by either using the full ClfA adhesin or by using only the A domain from ClfA which will screen for the same level of antibodies to ClfA. The A domain (as reflected in the specification and in other references cited in the specification) is a region within ClfA and within other staphylococcal adhesins which appears to give rise to the immunogenic reaction to the adhesin. Accordingly, the A domain is a subregion of the ClfA protein which appears to have the same immunogenic properties and can give rise to antibodies recognizing the full ClfA protein.

The Examiner's inquiry concerning the "second step" of the present method as reflected in the relevant dependent claims has been addressed above, and the claims have been amended to reflect that the second adhesin is also a staphylococcal

adhesin. For example, the second staphylococcal adhesin against which donors will be screened may be an staphylococcal Sdr protein such as SdrF, SdrG or SdrH. In these cases, donor plasma will thus first be screened for high antibody titers to ClfA (as in Claim 1), but will also be screened for high antibody titers to SdrF, SdrG or SdrH. These additional method steps will thus result in an immunoglobulin composition which will have a high titer to the Sdr protein in addition to a high titer to the ClfA protein. The resulting composition obtained by following the method of Claim 1 will have a high titer to ClfA only.

The Examiner's objection to Claim 37 resulted from the inadvertent reference in that Claim to Claim 1. This reference should have been to Claim 11, a composition claim, and this claim has now been corrected.

In short, all of the Examiner's objections are now traversed by virtue of the present arguments and/or amendments as made herein, and the claims are entirely proper under 35 U.S.C. § 112.

In the Official Action, the Examiner also rejected Claims 11, 22, 29 and 36 under 35 U.S.C. §102(e) as being anticipated by the Foster et al. US patent 6,008,341. The Examiner asserted that Foster disclosed immunoglobulin compositions directed to ClfA which had a titer of antibodies twice that of a control. This rejection is respectfully traversed in that Foster does not remotely disclose the present method steps nor the specific high titer human donor immunoglobulin compositions produced thereby. To the contrary, the passage in the Foster patent cited by the Examiner merely refers to the simple preparation of a generic immune sera (Cols. 7-8), and in fact is an immune sera obtained by immunizing rabbits and not to a human immunoglobulin having a high titer

to ClfA as in the claimed invention. Moreover, there is no disclosure or suggestion anywhere else in the patent of any steps necessary to obtain a human donor plasma immunoglobulin composition, much less the specific steps as required in the claims of the present patent. Accordingly, the Foster et al. patent does not disclose or suggest the presently claimed invention, and the Examiner's rejection on the basis of this reference, insofar as applied to the claims as amended, is respectfully traversed.

In the Official Action, the Examiner rejected Claims 11-22, 29-36 and 41 as being anticipated by Gristina US Patent 5,718,899 or 5,707,627. However, in making this rejection, the Examiner did not refer anywhere to any disclosure of screening for the specific ClfA adhesin as in the present claims, nor to any other specific adhesin, which is not surprising since neither of these Gristina patents discloses or remotely suggests such screening steps. Moreover, the Gristina patents do not disclose or suggest selecting donor plasma to high titer for <u>any</u> specific staphylococcal adhesin, much less the specific staphylococcal adhesins of the present claims. Even further, the reference made in Gristina to obtaining high titer donors as cited by the Examiner (col. 9, line 53) refers to donors having high titers to "specified bacteria or viruses" which clearly shows that there is no teaching or suggestion of obtaining donors with high titers to specific adhesins, such as ClfA, as set forth in the claims of the present application.

To the contrary, the Examiner's rejection of the claims seems to be based on the mistaken impression that because Gristina obtains an immunoglobulin composition based on elevated titers to bacteria such as *S. aureus*, this must inherently mean that there will some amount of antibodies which recognize ClfA in the final composition. However, it is in fact the case that <u>not all *S. aureus*</u> strains contain ClfA, and thus there

is no showing or suggestion in Gristina that these compositions will in fact have antibodies to ClfA at all. As shown in the attached article by Wickelhaus et al., *J. Clin. Microbiol.* 37(3):690-693 (1999), there are indeed a number of *S. aureus* strains that are clumping factor A-negative, and thus any compositions prepared on the basis of such staphylococcal strains will not contain antibody titers to ClfA, much less the higher titers as is the case in the present claims. Similarly, an *S. aureus* strain deficient in clumping factor was also identified in Nicholas et al., Infect. Immun. 67(7):3667-9 (1999). Accordingly, there is in fact no teaching or suggestion in the Gristina patents of the specific steps of the present invention nor the compositions produced thereby, and the Examiner's rejection on the basis of the Gristina patents is respectfully traversed and should be withdrawn.

The remaining rejections of the Examiner are similarly traversed in that none of the cited references, either singly or in combination, discloses or suggests the present invention, namely a method of obtaining a human immunoglobulin donor composition that has a higher than normal antibody titer to ClfA.

In the Official Action, the Examiner rejected Claims 11, 12-17, 22, 29, 30-36 and 41 on the basis of the Stephan US Patent 4,965,068 in light of WO 94/13310, WO 94/18327 and WO 95/34655. This rejections is respectfully traversed in that the Examiner recognized that this reference does not disclose or suggest any methods involving obtaining a human donor composition with elevated antibody titers to ClfA, but states that the strain of *S. aureus* used in Stephan would "inherently comprise bacterial pathogen expressed adhesins ClfA." See Official Action at Page 11. Once again, the Examiner must have assumed that any immunoglobulin raised against *S. aureus* must

inherently generate antibodies which recognize ClfA, which is not the case. In fact, as shown above in the Wickelhaus et al., many *S. aureus* strains do not contain ClfA and thus immunoglobulins raised against these strains would not recognize ClfA at all, much less in the higher than normal titers as is the case in Applicants' claims. Moreover, Sdr proteins such as SdrG are also not expressed in *S. aureus*.

In short, there is thus no teaching or suggestion in Stephan whatsoever of the creation of any human donor immunoglobulin having an antibody capable of recognizing ClfA or an Sdr protein such as SdrG, much less a method of obtaining such a composition having the necessary high titer as called for in Applicants' claims, nor is such a teaching or suggestion provided in the WO references cited by the Examiner. Accordingly, the Examiner's rejection on the basis of the Stephan reference, insofar as applied to the claims as amended, is respectfully traversed and should be withdrawn.

In the Official Action, the Examiner also rejected Claims 1-6, 12-17, 23-28, 30-35, 37 and 38-41 on the basis of the Fischer 1988 article in view of Wadstrom 1991 and the Foster et al. US patent 6,008,341. This rejection, insofar as applied to the claims as amended, is respectfully traversed is that there is no disclosure or suggestion in the cited references, either singly or in combination, of the specific method of obtaining an immunoglobulin composition of the present invention having higher than normal titers to ClfA. In the first place, as the Examiner recognizes, the Fischer reference does not make any reference or suggestion to develop an immunoglobulin with a high titer to ClfA, and had it been obvious to do so, there is no question that such a disclosure would have been made.

Moreover, there is nothing in Wadstrom or Foster which would add to Fischer to come up with the present invention. The Wadstrom reference merely relates to the possible use of staphylococcal adhesins to form vaccines, and merely reflects that the fact that staphylococcal adhesins were generally known to be used for vaccines at the time of the present invention. However, it was clearly not disclosed or suggested to make use of a specific adhesin, namely ClfA, and to screen donors so as to obtain an immunoglobulin composition with high titers to this specific adhesin as set forth in Applicants' claims.

Finally, the Examiner is incorrect with regard to the teachings of the Foster et al. US patent 6,008,341 in that this patent does not disclose or suggest the formulation of a human donor immunoglobulin composition having high titers to ClfA, much less a method of obtaining a human immunoglobulin having a higher than normal titer to ClfA as in the present claims. In fact, in the passage pointed to by the Examiner the preparation of an immune sera (Cols. 7-8), this indeed relates to an immune sera obtained by immunizing rabbits and not to a human immunoglobulin having a high titer to ClfA as in the claimed invention. Accordingly, the Foster et al. patent does not disclose or suggest any human donor immunoglobulin, much less the specific donor immunoglobulin composition of the present claims which has a high titer to ClfA.

In short, the simple fact is that nowhere in the references cited by the Examiner, either singly or in combination, is there a disclosure or suggestion of the steps necessary to obtain a human donor immunoglobulin composition having a higher titer to ClfA as set forth in the present claims, or a composition produced thereby, and

accordingly the Examiner's rejection on the basis of Fischer, Wadstrom and Foster is respectfully traversed and should be withdrawn.

Finally, in the Official Action, the Examiner rejected Claims 1-4, 38-40, 37, 11, 22, 29 and 36 on the basis of Hook et al. US patent 6,288,214. This rejection, insofar as applied to the claims as amended, is respectfully traversed. In the first place, according to the Examiner, the Hook patent teaches "the formulation of [a] selected donor immunoglobulin composition that comprises antibodies to ClfA and a second staphylococcal adhesin," but in fact, this is not the case, In the first place, Hook does not disclose or suggest a human donor immunoglobulin composition with **any** antibody titer to ClfA, much less a donor immunoglobulin with the high titer as set forth in the claims of the present application. Once again, as above with regard to the Foster reference, the passages the Examiner was pointing to do not disclose or suggest a human donor immunoglobulin composition in accordance with the claims but instead refer to the creation of rabbit antibodies, as shown for example in Section 5.3.9 of Column 61 of the Hook patent.

Moreover, the only reference to the production of antibodies in humans in the Hook patent is at Col. 30, line 52, but in fact this is with regard to "CBP", or the collagen binding proteins of the Hook patent and not ClfA. In addition, the patent does not disclose or suggest the preparation of a donor human immunoglobulin composition as in the present claims, nor is there a disclosure or suggestion of a method for producing any donor composition with a high titer to any adhesin, much less the particular human immunoglobulin composition of the present invention wherein a high titer to ClfA is

obtained. Accordingly, the Hook et al. patent is far afield from the present invention and clearly does not disclose or suggest the present claims.

In short, Applicants' invention relates to novel and unobvious methods for obtaining human donor immunoglobulin compositions which constitute a distinct improvement over less effective prior art methods leading to immunoglobulin products which have long been on the market and which have been made from pooled intravenous immunoglobulin obtained from donors not selected for high titers of antibodies to ClfA as in the present claims. As reflected above, no one prior to the present invention ever disclosed or suggested such human donor immunoglobulin compositions despite the fact that pooled immunoglobulin products had been prepared and utilized with varying levels of effectiveness for many years. Indeed, because there has long existed a need to develop more effective immunoglobulin compositions that can provide significantly improved treatment of staphylococcal infection as compared to the normal unselected pooled intravenous immunoglobulin products, Applicants' present method and the compositions obtained thereby represent a significant advance in the field of infection treatment and are clearly not disclosed or suggested in the myriad of references cited by the Examiner.

Applicants thus submit that none of the references cited by the Examiner, either singly or in combination, disclose or suggest the present invention, and that the Examiner's rejection on the basis of these references is respectfully traversed and should be withdrawn.

In light of the foregoing amendments and arguments, Applicants submit that the present application overcomes all prior objections, and is now in condition for immediate allowance. Such action is earnestly solicited.

Respectfully submitted,

March 18, 2003

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APPENDIX A

Marked-Up Replacement Paragraphs

Please replace the paragraphs as indicated with the following marked-up versions:

Please replace the paragraph starting at Page 29, line 31 as follows:

The SdrC, SdrD and SdrE proteins are related in primary sequence and structural organization to the ClfA and ClfB proteins and are localized on the cell surface. The SdrC, SdrD and SdrE proteins are cell wall-associated proteins, having a signal sequence at the N-terminus and an LPXTG motif, hydrophobic domain and positively charged residues at the C-terminus. Each also has an SD repeat containing region R of sufficient length to allow efficient expression of the ligand binding domain region A on the cell surface. With the A region of the SdrC, SdrD and SdrE proteins located on the cell surface, the proteins can interact with proteins in plasma, the extracellular matrix or with molecules on the surface of host cells. They share some limited amino acid sequence similarity with ClfA and ClfB. Additionally, SdrC, SdrD and SdrE also exhibit cation-dependent ligand binding to extracellular matrix proteins. For example, SdrC binds vitronectin and SrdE binds bone sialoprotein (BSP).

Please replace the paragraph starting at Page 30, line 12 as follows:

It has been discovered that in the A region of SrdC, SrdD, SrdE, ClfA and ClfB there is a highly conserved amino acid sequence that can be used to derive a consensus TYTFTDYVD (SEQ ID NO: 3) motif. The motif can be used in multicomponent vaccines to impart broad spectrum immunity to bacterial infections, and also can be used to produce monoclonal or polyclonal antibodies that impart broad

spectrum passive immunity. In an alternative embodiment, any combination of the variable sequence motif derived from the Sdr and Clf protein families, (T/I) (Y/F) (T/V) (F) (T) (D/N) (Y) (V) (D/N), can be used to impart immunity or produce protective antibodies.

Please replace the paragraph starting at Page 30, line 21 as follows:

ClfB, SdrC, SdrD and SdrE thus share a common consensus TYTFTDYVD (SEQ ID NO: 3) motif which overlaps the ligand binding/Ca2+ binding region of ClfA. Therefore the proteins interact with fibrinogen and other host components. ClfB, SdrC, SdrD and SdrE subdomains, depending on the protein, include subdomains A and B1-B5. Other information regarding extracellular matrix binding proteins has been disclosed in U.S. Application Ser. No. 09/200,650, incorporated herein by reference.

APPENDIX B

Clean Replacement Paragraphs

Please replace the paragraphs as indicated with the following clean versions of the new paragraphs:

Please replace the paragraph starting at Page 29, line 31 as follows:

The SdrC, SdrD and SdrE proteins are related in primary sequence and structural organization to the ClfA and ClfB proteins and are localized on the cell surface. The SdrC, SdrD and SdrE proteins are cell wall-associated proteins, having a signal sequence at the N-terminus and an LPXTG (SEQ ID NO: 2) motif, hydrophobic domain and positively charged residues at the C-terminus. Each also has an SD repeat containing region R of sufficient length to allow efficient expression of the ligand binding domain region A on the cell surface. With the A region of the SdrC, SdrD and SdrE proteins located on the cell surface, the proteins can interact with proteins in plasma, the extracellular matrix or with molecules on the surface of host cells. They share some limited amino acid sequence similarity with ClfA and ClfB. Additionally, SdrC, SdrD and SdrE also exhibit cation-dependent ligand binding to extracellular matrix proteins. For example, SdrC binds vitronectin and SrdE binds bone sialoprotein (BSP).

Please replace the paragraph starting at Page 30, line 12 as follows:

It has been discovered that in the A region of SrdC, SrdD, SrdE, ClfA and ClfB there is a highly conserved amino acid sequence that can be used to derive a consensus TYTFTDYVD (SEQ ID NO: 3) motif. The motif can be used in multicomponent vaccines to impart broad spectrum immunity to bacterial infections, and also can be used to produce monoclonal or polyclonal antibodies that impart broad

spectrum passive immunity. In an alternative embodiment, any combination of the variable sequence motif derived from the Sdr and Clf protein families, (T/I) (Y/F) (T/V) (F) (T) (D/N) (Y) (V) (D/N), can be used to impart immunity or produce protective antibodies.

Please replace the paragraph starting at Page 30, line 21 as follows:

ClfB, SdrC, SdrD and SdrE thus share a common consensus TYTFTDYVD (SEQ ID NO: 3) motif which overlaps the ligand binding/Ca2+ binding region of ClfA. Therefore the proteins interact with fibrinogen and other host components. ClfB, SdrC, SdrD and SdrE subdomains, depending on the protein, include subdomains A and B1-B5. Other information regarding extracellular matrix binding proteins has been disclosed in U.S. Application Ser. No. 09/200,650, incorporated herein by reference.

APPENDIX C

Marked-Up Amended Claims

The following is a marked-up version of the amended claims:

1. (Amended) A method of obtaining an immunoglobulin composition having a higher antibody titer ~~than a control antibody titer~~ to a staphylococcal clumping factor A (ClfA) adhesin ~~than that found in pooled intravenous immunoglobulin obtained~~ from unselected human donors comprising obtaining blood or plasma samples from human donors, identifying those blood or plasma samples from high-titer donors having the presence of an antibody titer to ClfA in an amount which is higher than that found in pooled intravenous immunoglobulin obtained from unselected donors, recovering blood or plasma from the identified high-titer donors, and treating the donor blood or plasma to obtain immunoglobulin in a purified state that has an antibody titer to ClfA in an amount which is higher than that found in intravenous immunoglobulin obtained from unselected donors.

4. (Amended) The method according to Claim 1 further comprising identifying those samples also having an antibody titer to a second staphylococcal adhesin which is higher than that found in pooled intravenous immunoglobulin obtained from unselected donors.

5. (Amended) The method according to Claim 4 wherein the second adhesin is a staphylococcal Sdr protein selected from the group consisting of SdrC, SdrD, SdrE, SdrF, SdrG, SdrH and clumping factor B (ClfB).

12. (Amended) A method of obtaining an immunoglobulin composition having a higher antibody titer to a staphylococcal ClfA adhesin than that found in pooled intravenous immunoglobulin obtained from unselected human donors ~~than normal antibody titer to a clumping factor A (ClfA) adhesin~~ comprising administering ClfA to a host donor in an amount sufficient so as to induce an antibody titer to ClfA in an amount which is higher than that found in pooled intravenous immunoglobulin obtained from unselected donors, recovering blood or plasma from the host donor, and treating the donor blood or plasma to obtain immunoglobulin in a purified state that has an antibody titer to ClfA which is higher than that found in pooled intravenous immunoglobulin obtained from unselected donors.

15. (Amended) The method according to Claim 12 further comprising administering a second staphylococcal adhesin to a host donor in an amount sufficient so as to induce an antibody titer to the second adhesin in an amount which is higher than that found in pooled intravenous immunoglobulin obtained from unselected donors.

37. (Amended) A method of immunizing patients so as to treat or prevent staphylococcal infection comprising administering an immunologically effective amount of the composition of claim ~~+~~11 to a patient in need of said treatment.

38. (Amended) The method according to Claim 4 wherein the second staphylococcal adhesin is selected from the group consisting of a fibronectin binding protein, a collagen binding protein, a fibrinogen binding protein, an elastin binding protein, an MHCII analogous protein, and other proteins that bind to extracellular matrix proteins.

39. (Amended) The method according to Claim 4 wherein the second adhesin is selected from the group consisting of proteins fibronectin binding protein A (FnBP-A), fibronectin binding protein B (FnBP-B), clumping factor protein B (ClfB), SdrC, SdrD, SdrE, SdrF, SdrG, SdrH, CNA, EbpS and MHCII.

APPENDIX D

Clean Replacement Claims

The following is a clean version of the amended claims:

1. (Amended) A method of obtaining an immunoglobulin composition having a higher antibody titer to a staphylococcal clumping factor A (ClfA) adhesin than that found in pooled intravenous immunoglobulin obtained from unselected human donors comprising obtaining blood or plasma samples from human donors, identifying those blood or plasma samples from high-titer donors having the presence of an antibody titer to ClfA in an amount which is higher than that found in pooled intravenous immunoglobulin obtained from unselected donors, recovering blood or plasma from the identified high-titer donors, and treating the donor blood or plasma to obtain immunoglobulin in a purified state that has an antibody titer to ClfA in an amount which is higher than that found in intravenous immunoglobulin obtained from unselected donors.

4. (Amended) The method according to Claim 1 further comprising identifying those samples also having an antibody titer to a second staphylococcal adhesin which is higher than that found in pooled intravenous immunoglobulin obtained from unselected donors.

5. (Amended) The method according to Claim 4 wherein the second adhesin is a staphylococcal Sdr protein selected from the group consisting of SdrC, SdrD, SdrE, SdrF, SdrG, SdrH and clumping factor B (ClfB).

12. (Amended) A method of obtaining an immunoglobulin composition having a higher antibody titer to a staphylococcal ClfA adhesin than that found in pooled intravenous immunoglobulin obtained from unselected human donors comprising administering ClfA to a host donor in an amount sufficient so as to induce an antibody titer to ClfA in an amount which is higher than that found in pooled intravenous immunoglobulin obtained from unselected donors, recovering blood or plasma from the host donor, and treating the donor blood or plasma to obtain immunoglobulin in a purified state that has an antibody titer to ClfA which is higher than that found in pooled intravenous immunoglobulin obtained from unselected donors.

15. (Amended) The method according to Claim 12 further comprising administering a second staphylococcal adhesin to a host donor in an amount sufficient so as to induce an antibody titer to the second adhesin in an amount which is higher than that found in pooled intravenous immunoglobulin obtained from unselected donors.

37. (Amended) A method of immunizing patients so as to treat or prevent staphylococcal infection comprising administering an immunologically effective amount of the composition of claim 11 to a patient in need of said treatment.

38. (Amended) The method according to Claim 4 wherein the second staphylococcal adhesin is selected from the group consisting of a fibronectin binding protein, a collagen binding protein, a fibrinogen binding protein, an elastin binding protein, an MHCII analogous protein, and other proteins that bind to extracellular matrix proteins.

39. (Amended) The method according to Claim 4 wherein the second adhesin is selected from the group consisting of proteins fibronectin binding protein A (FnBP-A), fibronectin binding protein B (FnBP-B), clumping factor protein B (ClfB), SdrC, SdrD, SdrE, SdrF, SdrG, SdrH, CNA, EbpS and MHCII.

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Rapid Detection of Epidemic Strains of Methicillin-Resistant *Staphylococcus aureus*

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Fifty methicillin-resistant *Staphylococcus aureus* (MRSA) initial isolates obtained from patients hospitalized in the orthopedic clinic of the Frankfurt University Hospital and 150 methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates were investigated in this study to determine whether the Slidex Staph-Kit is capable of differentiating between MRSA and MSSA owing to its unique performance characteristics. The Slidex Staph-Kit is a combined latex hemagglutination test designed to detect clumping factor, protein A, and a specific surface immunogen for *S. aureus*. Clumping factor-positive strains cause erythrocytes sensitized with fibrinogen to hemagglutinate, thereby resulting in visible red clumps. *S. aureus* strains deficient in clumping factor agglutinate latex particles sensitized with specific antibodies against surface proteins of *S. aureus*, thereby resulting in visible white clumps. Our results demonstrate that white clumping has a 99% specificity as well as a 98% positive predictive value for MRSA. Clumping factor-negative MRSA, which have been reported to occur in several countries, are epidemic in the Frankfurt area and account for 80% of all MRSA initial isolates in the orthopedic clinic of the Frankfurt University Hospital. Genotyping of all MRSA isolates by macrorestriction analysis of chromosomal DNA revealed that 83% of clumping factor-negative MRSA are closely related to the "southern-German" epidemic strain. This is the first study demonstrating the Slidex Staph-Kit's capability for identifying epidemic clumping factor-negative *S. aureus* strains as methicillin resistant even prior to antimicrobial susceptibility testing.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were initially described in 1961 and emerged in the 1980s as a major epidemiological problem in hospital settings (8, 16). MRSA strains with widely different properties have now become endemic in hospitals and are considered important nosocomial pathogens (14, 24, 26). Methicillin resistance rates differ markedly among countries and range from less than 1% in Scandinavia to 60% in Japan (11, 27).

Accordingly, rapid detection of MRSA is crucial for purposes of initiating hygienic measures and preventing further spread of the pathogen. Commercially produced agglutination kits are available for the rapid identification of *S. aureus* by clinical laboratories. Nonetheless, virtually all of them have the drawback that additional time-consuming antimicrobial susceptibility testing generally is required in order to detect methicillin resistance in *S. aureus*.

The aim of this study was to assess the capability and specificity with which the Slidex Staph-Kit differentiates between MRSA and methicillin-susceptible *S. aureus* (MSSA).

MATERIALS AND METHODS

Bacterial strains. A total of 50 MRSA isolates and 150 MSSA isolates were investigated in this study. The MRSA initial isolates were cultured from clinical specimens obtained from the orthopedic clinic of the Frankfurt University Hospital between 1993 and 1997. All isolates had been classified as MRSA during routine investigations and were stored in stock cultures prior to the study, as described previously (29). The MSSA isolates were cultured consecutively from clinical specimens obtained from the Frankfurt University Hospital. *S. aureus* ATCC 25923 was employed as the reference strain.

Agglutination kits and further characterization. The Slidex Staph-Kit (bioMerieux Vitek, Inc., Hazelwood, Mo.) is a latex and erythrocyte combination agglutination system for the detection of clumping factor, protein A, and other specific immunogens of *S. aureus*. The Staphylase test (Oxoid, Basingstoke, England) is an erythrocyte agglutination kit for the detection of clumping factor. Both agglutination tests were performed according to the manufacturers' instructions. A tube coagulase test was performed on each isolate. Plasma coagulation was tested with rabbit plasma (Difco, Detroit, Mich.), and the results were read after 2, 4, 6, and 24 h at 37°C. All isolates were screened for hyaluronidase activity by testing the decapsulation reaction on a cross-inoculated streak of a mucous strain of *Streptococcus equi* (9, 19).

Detection of methicillin resistance. All of the *S. aureus* isolates were cultured on Mueller-Hinton agar supplemented with 6 μg of oxacillin/ml and 4% NaCl (Biotest, Heidelberg, Germany) (13, 20). The plates were incubated at 30°C for 48 h and examined for evidence of growth to detect resistance phenotypically. The presence of the *mecA* gene was proved for all strains by means of PCR. Purification of bacterial DNA was carried out with the QIAmp tissue kit (Qiagen, Hilden, Germany) in accordance with the manufacturers' instructions. Amplification of the *mecA* gene was performed with the primers *mec*A1 (5'-AAA ATC GAT GGT AAA GGT TGG C) and *mec*A2 (5'-AGT TCT GCA GTA CCG GAT TTG C), yielding a PCR product of 533 bp (15). DNA amplification was carried out for 30 cycles as previously described (25): denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 5 min. The PCR product was cleaved by the restriction enzyme *Hha*I (New England BioLabs, Schwalbach, Germany) to confirm the specificity of the *mecA* primers (25). Electrophoresis of DNA was carried out in 2% agarose gel, which was then stained with ethidium bromide and photographed under UV light.

Genotyping. All MRSA isolates were analyzed by pulsed-field gel electrophoresis (PFGE) as described previously (29). Briefly, genomic DNA was prepared in low-melting-point agarose plugs and digested with *Sma*I restriction enzyme (New England BioLabs). Electrophoresis was performed on the CHEF-DR III (Bio-Rad Laboratories, Richmond, Calif.) apparatus. A constant voltage of 6 V/cm was applied, with an increasing pulse time of 5 to 50 s over a period of 22 h in order to separate DNA fragments. Computer-aided analysis of DNA fragment patterns was performed with the DNA fingerprint analysis software Wincam 2.2 (Cybertech, Berlin, Germany).

RESULTS

Strain characterization. Coagulase production and hyaluronidase activity was demonstrated for all *S. aureus* isolates in

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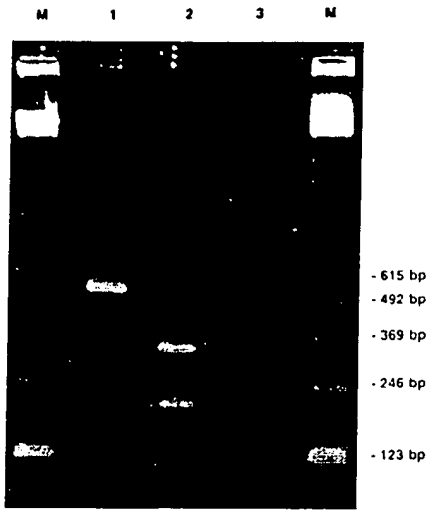

FIG. 1. Agarose gel electrophoresis of *mecA* PCR product. Lanes: M, size markers; 1, *mecA*-positive *S. aureus* strain; 2, restriction of amplified DNA with *Hha*I; 3, *mecA*-negative *S. aureus* reference strain ATCC 25923.

this study. Methicillin resistance was proved genotypically for all isolates by amplification of the *mecA* gene (Fig. 1) as well as phenotypically by growth on Mueller-Hinton agar supplemented with 6 μg of oxacillin/ml and 4% NaCl.

Genotyping. Macrorestriction analysis of 50 MRSA initial isolates obtained from the orthopedic clinic of the Frankfurt University Hospital in a 5-year period revealed 18 different genotypes (Fig. 2). Fifty percent of all isolates belonged to type 1, and strongly corresponding restriction fragment patterns indicating close clonal relatedness between MRSA types 1, 7, 71, and 72 were obvious (Fig. 3).

Agglutination performance. Table 1 compares the performance of the Slidex Staph-Kit with that of the Staphylase agglutination test. It could be demonstrated that all MRSA initial isolates closely related to the epidemic strain, as well as isolates belonging to MRSA types 6, 76, 82, and 83, exhibited white-clumping behavior (Fig. 4). In every instance where the Slidex Staph-Kit exhibited white clumping the Staphylase agglutination test was negative, which indicates that these strains were at least phenotypically clumping factor negative. In contrast, 149 of 150 MSSA isolates classified during routine investigation were determined to be clumping factor positive, i.e., they exhibited red-clumping behavior (Fig. 5).

DISCUSSION

Rapid and accurate identification of methicillin resistance in *S. aureus* is of ongoing clinical importance for controlling the spread of this pathogen within hospital settings. The plasma coagulase test is generally acknowledged as the "gold standard" for the identification of *S. aureus*. Nevertheless, the use of commercial agglutination kits for identifying *S. aureus* is widespread in clinical microbiological laboratories, since these tests are easy to perform and the results are available within minutes. Whereas the first-generation agglutination kits are capable of detecting clumping factor and/or protein A for *S. aureus* strains while failing to identify certain MRSA strains (3, 22), second-generation agglutination kits, such as the Slidex Staph-Kit, possess the additional feature of being able to detect specific surface antigens for *S. aureus*. Numerous studies have been published confirming the high sensitivity and specificity of these second-generation kits for the identification of MSSA as well as MRSA (1, 4–6, 10, 17, 23, 30). Definitive detection of methicillin resistance, however, still requires time-consuming antimicrobial susceptibility testing.

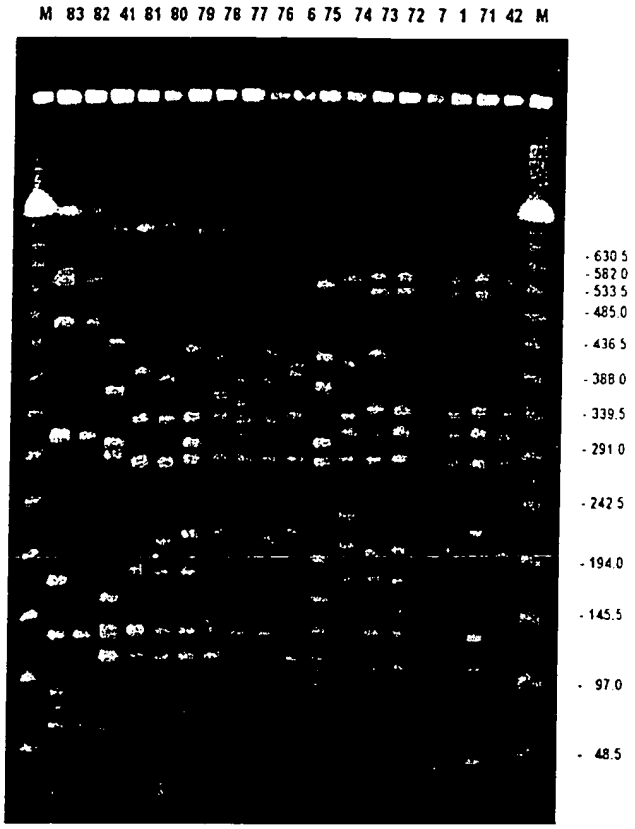

FIG. 2. PFGE patterns of *Sma*I digests of total DNA from representatives of each pulsotype (indicated by numbers above lanes). Lanes M, size markers. Molecular sizes (in kilobases) are indicated on the right.

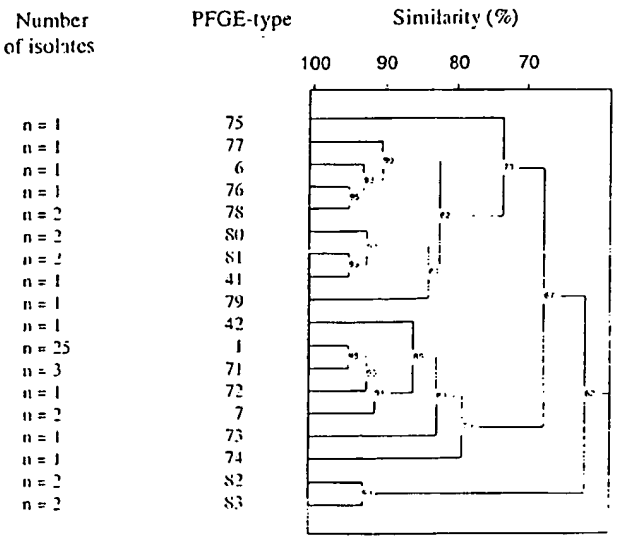

FIG. 3. Computer-aided analysis of different PFGE types, indicating clonal relatedness.

TABLE 1. Performance of the Slidex Staph-Kit and Staphylase test for the identification of MRSA and MSSA

Organism	Type	*n*	Results[a]	
			Slidex Staph-Kit	Staphylase test
MRSA	1	25	W	–
	7	2	W	–
	71	3	W	–
	42	1	W	–
	72	1	W	–
	73	1	W	–
	74	1	W	–
	75	1	R	R
	6	1	W	
	76	1	W	–
	77	1	R	R
	78	2	R	R
	79	1	R	R
	80	2	R	R
	81	2	R	R
	41	1	R	R
	83	2	W	–
	82	2	W	–
MSSA		150	R/W[b]	ND

[a] W, white clumping; R, red clumping; –, no clumping; ND, not determined.
[b] R/W, both R (n = 149) and W (n = 1).

This is the first study demonstrating that the Slidex Staph-Kit is a reliable and, above all, rapid method for identifying epidemic clumping factor-negative MRSA even prior to antimicrobial susceptibility testing. Owing to its unique design as a combined latex and hemagglutination test, it reacts, in contrast to all the other latex agglutination kits, in two different ways. Red clumps indicate the presence of clumping factor, with which *S. aureus* causes erythrocytes sensitized with fibrinogen to agglutinate (Fig. 5). White clumps, on the other hand, signal both the absence of clumping factor and the presence of other immunogens, which trigger the agglutination of the latex particles (Fig. 4). This study shows that white clumping has a 99% specificity and a 98% positive predictive value for the detection of methicillin resistance in *S. aureus*. Fifty percent of all initial isolates obtained from the orthopedic clinic of the Frankfurt University Hospital and more than 60% of all initial isolates in the Frankfurt metropolitan area, including six community hospitals and the University Hospital (29), belong to one genotype, which has been identified by the Robert Koch Institute, the German national institute of infectious diseases, as the so-called "southern-German" epidemic strain. This epidemic strain is clumping factor negative and exhibits white-clumping behavior. Furthermore, it is not only epidemic in the south but is also widespread in the northern parts of Germany (22, 31). Since bacteria do not respect international boundaries, it came as no surprise when epidemic MRSA strains kindly sent to our institution from Slovakia and Italy exhibited the same southern-German genotype and showed white-clumping behavior (data not shown). As demonstrated by PFGE in this study, it is not only the southern-German epidemic strain which is clumping factor negative; other MRSA genotypes also manifest this typical agglutination characteristic.

The Slidex Staph-Kit, of course, is neither designed nor licensed to detect resistance, and as such, white clumping can be regarded only as a clue to methicillin resistance. Nonetheless, to the extent that the white clumping serves as a strong indicator for MRSA, rapid tests can be conducted which confirm methicillin resistance and, upon request, species identity within 4 hours. This validation can be achieved genotypically, for example, by multiplex PCR, a method that enables the detection of the *mecA* gene and a species-specific gene for *S. aureus* within 5 to 6 h (2, 21). Quicker and less labor intensive is the phenotypical verification of MRSA with the BBL Crystal MRSA ID system from Becton Dickinson, which can be performed within 4 h (12, 18, 28). Finally, the phenotypical test for plasma coagulation can be performed to confirm spe-

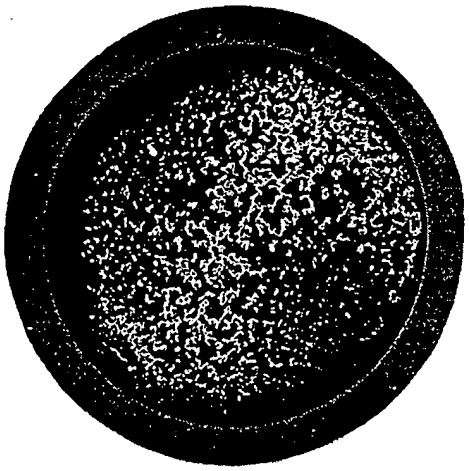

FIG. 4. Performance characteristics of Slidex Staph-Kit: white clumping. Magnification, ca. ×2.

FIG. 5. Performance characteristics of Slidex Staph-Kit: red clumping. Magnification, ca. ×2.5.

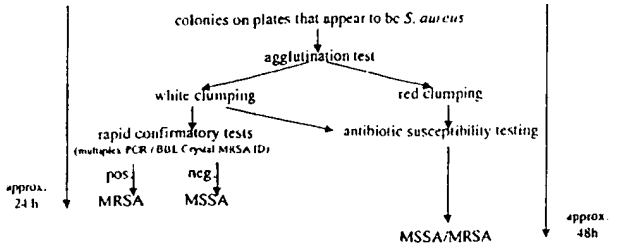

FIG. 6. Procedure for the rapid identification of clumping factor-negative MRSA with the Slidex Staph-Kit.

cies specificity, analogous to the genotypic confirmation by multiplex PCR (7) (Fig. 6).

The results of this study, as well as the fact that a modern second-generation agglutination test was required owing to the rising incidence of clumping factor-negative MRSA, indicate that all clumping factor-negative MRSA strains are capable of being recognized by the Slidex Staph-Kit with its differentiated agglutination characteristics. In view of these findings, the Slidex Staph-Kit is a valuable tool in clinical laboratories with a frequent occurrence of clumping factor-negative MRSA.

In conclusion, this study confirms the Slidex Staph-Kit's ability within the context of the clinical microbiological laboratory to pinpoint certain epidemic MRSA strains 24 h prior to the final results provided by antimicrobial susceptibility testing. In turn, the initiation of effective infection control measures may reduce the spread of this pathogen.

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